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Cytogenetic effects of tobacco smoke exposure among involuntary smokers

Marja Sorsa, Kirsti Husgafvel-Pursiainen, Hilkka Järventaus, Kaija Koskimies, Helena Salo and Heini Vainio

Institute of Occupational Health, SF-00250 Helsinki (Finland)

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Summary

Tobacco smoke is highly genotoxic and produces chromosomal damage in several experimental systems. Active smokers have been shown to have an increased prevalence of somatic chromosome damage in their peripheral blood lymphocytes: this is seen in most cases as an increased sister-chromatid exchange (SCE) frequency and often also as increased structural chromosome aberrations (CAs). Among passive smokers, in association with exposure to environmental tobacco smoke, no such induction of chromosomal damage has been documented. In the present paper we report negative results on induction of chromosomal damage in 2 separate groups of intensive involuntary exposure to tobacco smoke, non-smoking restaurant personnel and newborn children of smoking mothers. While significant exposure in these groups is clearly seen in biochemical intake markers, e.g. cotinine and thiocyanate values in plasma, the conventional cytogenetic parameters, structural chromosome aberrations and sister-chromatid exchanges, are unable to detect the low exposures of involuntary smokers.

Most involuntary smoking occurs through exposure to environmental tobacco smoke (ETS), i.e., passive smoking. The chemical and biological characteristics of ETS have been discussed in this volume by Löfroth, Claxton and collaborators. Transplacental exposure of the unborn child is also, in fact, involuntary even if the chemical composition of the exposing agent, tobacco smoke, is different from ETS; it mainly consists of the transplacental components of main-stream smoke inhaled by the actively smoking mother.

The well-documented genotoxic character of tobacco smoke condensates and all forms of tobacco smoke, main-stream, side-stream and environmental tobacco smoke in experimental conditions (see e.g., IARC, 1986; Claxton et al., this volume), also makes the exposure measures that are specific to the genotoxic character applicable in the natural exposure situation. In this report, we especially discuss the use of cytogenetic exposure measures, i.e., structural chromosome aberrations (CAs) and sister-chromatid exchanges (SCEs) in peripheral blood lymphocytes, to detect involuntary exposure to tobacco smoke in heavily exposed passive smokers and in newborn babies of smoking mothers.

Correspondence: Dr. Marja Sorsa, Institute of Occupational Health, Topeliuksenkatu 41 a A, SF-00250 Helsinki (Finland).

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Subjects and methods

The occupationally exposed group comprised 16 non-smoking waiters and 9 smoking waiters working in a night restaurant where there was no restriction in smoking. As controls, 7 non-smoking persons without obvious recent or past exposure to environmental tobacco smoke were included. A detailed description of the work-site restaurant (restaurant B) of the subjects was given in an earlier publication (Husgafvel-Pursiainen et al., 1986). The non-cytogenetic exposure parameters of the subjects (together with personnel from 2 other restaurants) have been described (e.g., cotinine, thiocyanate, carboxyhaemoglobin) in Husgafvel-Pursiainen et al. (1987) and the detailed results of the sister-chromatid exchange analysis by Husgafvel-Pursiainen (1987).

The present paper reports the analysis of chromosome aberrations in these same subjects and discusses the individual concordance of the 2 cytogenetic parameters, CAs and SCEs.

For the CA analysis the whole-blood microculture method was used with a 50-h culture time (see Mäki-Paakkanen et al., 1980 for details). The scoring was performed on coded slides by an experienced analyst counting 100 metaphases per subject.

The SCE frequencies of the transplacentally exposed group were studied from cord-blood samples taken at delivery. To avoid methodological variation between culture periods, a large batch of culture medium, including bromodeoxyuridine (20 μM; Calbiochem), phytohemagglutinin (1%; Wellcome), penicillin (100 units/ml) and streptomycin (100 μg/ml) in RPMI 1640 (Gibco) supplemented with 15% fetal calf serum (Gibco) was prepared for the whole study and stored at $-20\,^{\circ}C$. The sister-chromatid exchange analyses were performed on duplicate cultures harvested and stained according to standard procedures (see Husgafvel-Pursiainen, 1987), scoring on codes, 50 second metaphases per individual.

The mothers were interviewed for their smoking habits and possible passive exposure a few days after the delivery. The group consisted of 17 actively smoking mothers and their newborn babies and 25 non-smoking mothers and newborns; only 7 of the non-smoking mothers described some exposure to environmental tobacco smoke. The biochemical intake markers measured from both the maternal and the cord-blood plasma samples were cotinine and thiocyanate; the analytical results and their discussion are reported separately (Sorsa et al., in preparation). In the present paper we discuss the findings of maternal–child correlations of SCEs in association with the smoking status of the mother.

Results and discussion

Chromosomal damage in passively exposed restaurant personnel

Non-smoking personnel working in indoor restaurants without restrictions of smoking of the public or personnel probably represents one of the groups most heavily exposed to ETS at work. The environmental monitoring data, including analysis of polyaromatic compounds, total particulate matter and genotoxic activity of particulate samples, from typical night restaurant show high levels of contamination of the indoor air (Husgafvel-Pursiainen et al., 1986). The biochemical intake markers of tobacco smoke, i.e., cotinine and thiocyanate, also show significantly increased values in the passively exposed restaurant personnel as compared with non-exposed persons (Husgafvel-Pursiainen et al., 1987). Still, these intake markers are only a few percent of the values found in the actively smoking group of waiters (Fig. 1).

Both chromosome aberrations and SCEs were analysed in a total of 32 subjects (Table 1, Fig. 2). No significant differences were seen between the

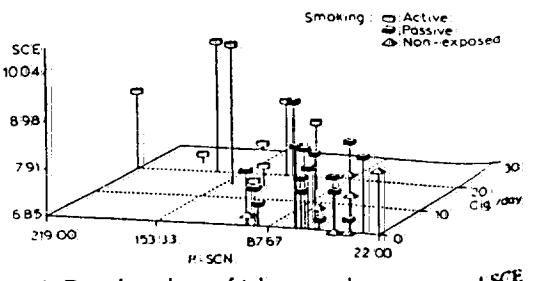

Fig. 1. Dose dependence of tobacco smoke exposure and SCE frequency among restaurant personnel, as evaluated on the basis of individual values in mean SCE/cell, thiocyanates (μmole/l) in plasma (P-SCN) and number of cigarettes smoked daily.

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TABLE 1

MEAN LYMPHOCYTE SCEs AND MEAN PERCENTAGE OF LYMPHOCYTES WITH CHROMOSOME ABERRATIONS IN DIFFERENT SMOKING CATEGORIES

Smoking status	Number of subjects	Mean SCEs /cell (± SD)	% aberrant cells (incl. gaps; ± SD)
Active smokers	9	8.21 ± 1.19	5.0 ± 1.7
Non-smokers			
Passively exposed ex-smokers	9	8.14 ± 0.76	4.6 ± 3.1
Passively exposed never-smokers	7	8.08 ± 0.62	2.7 ± 1.8
Non-exposed	7	7.47 ± 0.59	3.7 ± 1.6

groups or subgroups in the 2 parameters, neither was there any correlation at the individual level. In the total material, SCEs of the smoking waiters were significantly increased in comparison to non-smoking persons in the study (Husgafvel-Pursiainen, 1987). However, a clear trend in the prevalence of chromosome aberrations, especially in chromosome-type aberrations, is seen in the group of ex-smokers (mean non-smoking time after cessation of smoking was 9.6 ± 6.8 years; range 1–23 years) and smokers (Table 2). The difference is significant ($P < 0.05$) when all never-smokers (n = 17) are compared with current and ex-smokers (n = 18). However, in the larger population (Husgafvel-Pursiainen et al., 1987), neither cotinine nor SCE values of passively exposed ex-smokers (U-cot mean 52 ± 27 ng/ml, SCE mean 7.2 ± 1.3, n = 12) differed significantly from the values of never-smokers (U-cot mean 60 ± 44 ng/ml, SCE mean 8.6 ± 1.1, n = 15).

The significance of chromosome-type aberrations and rearrangements has been stressed earlier, since they are independent of age and sex and are positively correlated with the duration of smoking (Vijayalaxmi and Evans, 1982; Obe et al., 1984; Littlefield and Joiner, 1986). The result also indicates the persistence of lesions leading to chromosome-type aberrations in persons who stopped smoking years ago. In comparison, the SCEs in peripheral lymphocytes of smokers decrease to normal in a few months after stopping smoking (Sarto et al., 1987).

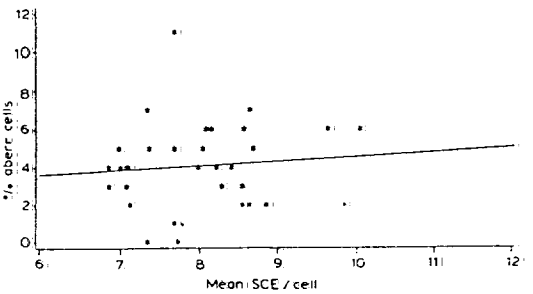

Fig. 2. Relationship of structural chromosome aberrations and SCEs. ($r = 0.089$, non-significant) among the group of restaurant personnel (see Fig. 1).

Fetal exposure to tobacco smoke

The hazards of maternal smoking to the normal development of the fetus have long been known (see e.g., National Research Council, 1986). Risks of spontaneous abortion, preterm births, prenatal deaths and low full-term birth weights have been associated with maternal smoking during pregnancy (Surgeon General, 1986). A recent case–control study suggested an association between maternal smoking and increased risk of childhood malignancies (Stjernfeldt et al., 1986). Lowering of birthweight has also been associated with heavy passive exposure of the mother to ETS (Martin and Bracken, 1986; Rubin et al., 1987), and fetal exposure caused by maternal passive smoking has

TABLE 2

NUMBER OF CHROMOSOME-TYPE ABERRATIONS IN SMOKERS, EX-SMOKERS AND NEVER-SMOKERS [a]

	Smokers	Ex-smokers	Never-smokers
Dicentrics	1/900	3/900	1/1400
Other rearrangements	3/900	2/900	1/1400
Breaks	6/900	9/900	7/1400
Total	10/900	14/900	9/1400

[a] Number of aberrant cells/cells analyzed.

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been documented by the detection of cotinine in amniotic fluid samples (Andersen et all, 1982).

In the present study, we report the sister-chromatid exchange results in cord-blood samples taken during delivery of actively and passively exposed smokers and non-exposed non-smokers.

Biochemical intake markers measured from maternal peripheral blood just before delivery and from cord blood just after birth of the child showed transplacental concentrations of these tobacco smoke constituents to be at nearly the same level as in the mother. The correlation coefficient of maternal–cord-blood plasma cotinine was $r = 0.81$ ($P < 0.001$; 11 pairs) and for plasma thiocyanate $r = 0.92$ ($P < 0.001$; 17 pairs).

As expected on the basis of earlier studies, smoking mothers had significantly ($P < 0.01$) increased SCE values (range 7.5–10.8; mean 9.0 ± 0.9; $n = 17$) as compared with non-smoking mothers (range 6.7–8.9; mean 8.1 ± 0.9; $n = 25$). A dose–effect correlation was seen both for plasma thiocyanate value and for the number of cigarettes smoked daily (Fig. 3A).

Only 7 of the non-smoking mothers said in the personal interview that they had been exposed to ETS because of spouse's smoking. However, no significant increase of SCEs was detected in this group of passive smokers; the mean was 8.4 ± 1.0 as compared with 7.9 ± 0.9 of the non-exposed non-smoking mothers ($n = 18$).

On the basis of the biochemical intake markers, however, the small group of passively exposed persons were exposed at their homes only minimally. All of the mothers had been out of occupational ETS exposure about one month before delivery during their maternity leave.

The SCE rate in the cord-blood samples was significantly lower (mean $= 6.0 \pm 0.5$; $n = 43$) than in maternal samples (mean $= 8.4 \pm 0.9$; $n = 42$). We reported earlier also (Husgafvel-Pursiainen et all, 1980) that young children (mean age 1.5 years) have significantly lower SCE rates than adults. In earlier studies of smoking mothers and their newborn children no effect of smoking on SCEs was observed in maternal blood, while these studies also reported lower mean SCE rates in cord-blood samples (Ardito et al., 1980; Seshadri et al., 1982). A possible source of discrepancy in the maternal SCE values of smokers vs. non-smokers is the

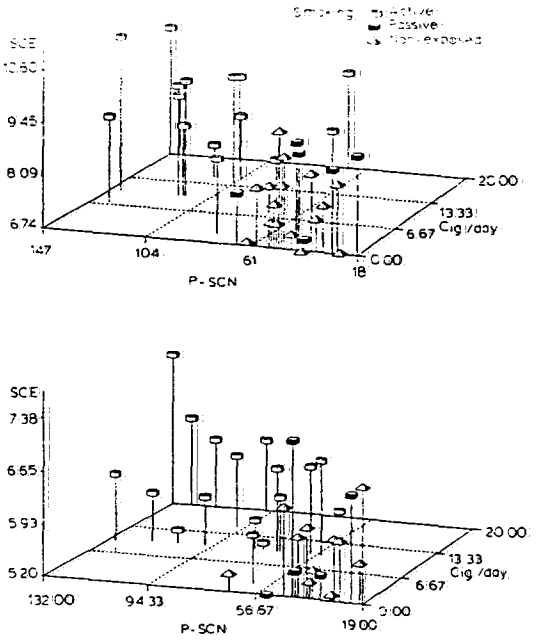

Fig. 3. Association of individual mean SCE to thiocyanate concentration (μmole/l) in plasma (P-SCN) and maternal smoking (number of cigarettes/day) in groups of smoking and non-smoking mothers (A) and newborn children (B); in A the P-SCN and SCE were determined from peripheral blood and in B from cord blood.

bromodeoxyuridine concentration used during culturing, as shown by Lundgren et al. (1987).

In transplacentally exposed children no significant effect of maternal smoking on SCEs was observed (mean 6.1 ± 0.5 in babies of smoking mothers vs. 5.9 ± 0.5 in babies of non-smoking mothers). In the individual values, however, a clear trend of heavy maternal smoking can be seen; children with the highest plasma thiocyanate values tend to have higher SCE rates than children of mild smokers and non-smokers (Fig. 3B), although this difference is not statistically significant. Neither was there any correlation between maternal SCE value and cord-blood value, even though cotinine and thiocyanate concentrations in plasma showed highly significant correlations between maternal and cord-blood samples.

Several hypotheses can be presented to explain the low SCE rate in cord-blood lymphocytes: either the lymphocyte population responding to the

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mitogen stimulus is different in its SCE response in children and adults, or transplacental exposure to exogenous SCE-inducing agents differs from the maternal situation with regard to effective concentrations and long enough exposure time during the fetal period.

Recently accumulated evidence associates involuntary exposure to environmental tobacco smoke with health effects in non-smokers; this has been most clearly shown for respiratory organ infections among children exposed to parental smoking and for a small increase of lung cancer risk among non-smoker spouses exposed to ETS caused by the other spouse's smoking (Surgeon General, 1986; National Research Council, 1986). The biochemical intake markers usually show passive smokers' exposure to be only a few percent of the values found among active smokers (see e.g., Jarvis et al., 1984, 1985; Sorsa et al., 1985). In newborn children exposed transplacentally, the biochemical intake markers show values at nearly the same level as in their mothers; still, the fetal exposure time is only a few months.

The cytogenetic damage, well documented among active smokers (see e.g., IARC, 1986), cannot be shown to be associated with involuntary exposure to tobacco smoke in either of the two exposure situations studied, occupational or transplacental. The obvious insensitivity of the cytogenetic parameters may partly be due to their unspecificity in relation to any single compound. Also, tobacco smoke exposure may be confounded by other potentially genotoxic agents in the environment.

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